

CHARGE NUMBER: 2525

PROJECT TITLE: Chemistry and Isolation of Tobacco Constituents

PROJECT LEADER: H. J. Grubbs

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An additional harvest of yellowed Kudya leaves (*Pueraria lobata*) has been carried out.<sup>1</sup> Using the methods previously reported, a steam distillation followed by methylene chloride extraction of the distillate has resulted in an essential oil. This essential oil was examined by capillary gas chromatography<sup>2</sup> and shown to be quite different from the essential oil of the green leaf. A number of tobacco identical flavorants including damascone were identified. Harvest of additional leaf material will continue throughout the growth season with isolation and characterization of the essential oil.<sup>3</sup>

It has been well established that a number of amino acid-sugar compounds do occur naturally in bright tobacco.<sup>4</sup> The isolation and quantitation of these compounds as a function of the natural ageing of flue-cured leaf tobaccos has been studied extensively.<sup>5</sup> The content of 1-deoxy-1-proline fructose, the most prominent component of the amino acid-sugar compounds, increased during the initial period of storage. Only after two years of ageing did the level begin to decline.

The preparation and isolation of this tobacco identical Amadori compound is complete.<sup>6</sup> Complete chromatographic and spectral characterization of this compound is currently under investigation. Concurrently, the preparation of the corresponding asparagine and valine tobacco identical Amadori compounds is underway. Pyrolysis studies with these compounds will aid in developing an understanding of their contribution to tobacco flavor and aroma.

At the request of Flavor Development, a seventy gram sample of highly purified WS-14 has been prepared and delivered for use as a reference standard. This material is currently being used to establish rigid specifications for purchase agreements with potential commercial suppliers of WS-14.<sup>7</sup>

In a collaborative effort with J. S. Long and Project 1101, we have initiated an isolation of azadirachtin.<sup>8</sup> Two kilos of neem needs (*Ayadirachta indica*) have been worked up in a new isolation scheme.<sup>9</sup> Grinding of seeds in solvent, lipid removal, and column chromatography has resulted in a fraction of crude azadirachtin. Spectroscopic and chromatographic examination of this material is currently in progress. Purified samples of this fraction will be supplied to J. S. Long for assay as an insect antifeedant.

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Several new methods of isolation and quantitation of tobacco alkaloids have been developed. In conjunction with Project 2506, the alkaloid content and alkaloid distribution (mass and radiochemical) has been determined for the four different cultivars of  $^{14}\text{CO}_2$  chamber grown tobaccos.<sup>10</sup> With the arrival of one additional minor alkaloid standard, the quantitative data will be available for publication. This procedure, as well as an auxillary HPLC procedure, is currently being developed as a preparative method.<sup>11</sup>

References:

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